

IN THE CLAIMS

The status of each claim is provided below.

Claims 1-109: (Canceled).

110. (Currently Amended) A method of determining the initial amounts of individual species of a target gene, comprising:

amplifying a target gene and monitoring the amplification by real-time PCR;
performing a polymorphous analysis with respect to the amplified target gene to determine a polymorphous composition ratio of individual species of the target gene; and
determining the initial amount of the target gene from the percentage of a change in the intensity of fluorescence occurring as a result of hybridization of the nucleic acid ~~probe~~ probe to ~~and~~ the amplified target gene; and

determining the initial amounts of individual species of the target gene by multiplying the initial amount of the target gene by said polymorphous composition ratio of individual species of the target gene,

wherein the real-time PCR is accomplished with a nucleic acid probe,
wherein the probe comprises a single-stranded oligonucleotide capable of hybridizing to the target gene,

wherein the probe is labeled with a fluorescent dye and a quencher substance,
wherein the oligonucleotide is labeled with the fluorescent dye and the quencher substance such that the intensity of fluorescence in a hybridization reaction system increases when the probe is hybridized with the target gene, and

wherein the oligonucleotide forms no stem-loop structure between bases at positions where the oligonucleotide is labeled with the fluorescent dye and the quencher substance.

Claims 111-114: (Canceled).

115. (Previously Presented) The method of Claim 110, wherein the polymorphous analysis is T-RELP (terminal restriction fragment length polymorphism), RFLP (restriction fragment length polymorphism), SSCP (single strand conformation) or CFLP (cleavage fragment length polymorphism).

Claim 116: (Canceled).

117. (Previously Presented) The method of Claim 110, wherein the single-stranded oligonucleotide is labeled on the same nucleotide thereof with the fluorescent dye and the quencher substance.

Claim 118: (Canceled).

119. (Previously Presented) The method of Claim 110, wherein the distance between the bases at the positions where the oligonucleotide is labeled with the fluorescent dye and quencher substance, respectively, is 1 to 20 bases.

Claim 120: (Canceled).

121. (Previously Presented) The method of Claim 110, wherein the probe is labeled at a 3' end thereof with the fluorescent dye.

Claim 122: (Canceled).

123. (Previously Presented) The method of Claim 110, wherein the probe is labeled at a 5' end thereof with the fluorescent dye.

124. (Previously Presented) The method of Claim 110, wherein the probe has G or C as a 5' end base and is labeled at the 5' end thereof with the fluorescent dye.

125. (Previously Presented) The method of Claim 110, wherein a hydroxyl group on a 3' carbon of ribose or deoxyribose at the 3' end or a hydroxyl group on a 3' or 2' carbon of ribose at the 3' end has been phosphorylated.

Claims 126-127: (Canceled).

128. (Previously Presented) The method of Claim 110, wherein the probe is labeled at a 5' end phosphate group and/or a 3' end phosphate group thereof with the fluorescent dye.

Claim 129: (Canceled).

130. (Previously Presented) The method of Claim 110, wherein the oligonucleotide of the probe is a chemically-modified nucleic acid.

Claim 131: (Canceled).

132. (Previously Presented) The method of Claim 130, wherein the chemically-modified nucleic acid is 2'-O-methyloligonucleotide, 2'-O-ethyloligonucleotide, 2'-O-butyloligonucleotide, 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

133. (Previously Presented) The method of Claim 110, wherein the oligonucleotide of the probe is a chimeric oligonucleotide which comprises a ribonucleotide and a deoxyribonucleotide.

Claim 134: (Canceled).

135. (Previously Presented) The method of Claim 133, wherein the chimeric oligonucleotide comprises 2'-O-methyloligonucleotide, 2'-ethyloligonucleotide, 2'-O-butyloligonucleotide 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

Claims 136-142: (Canceled).

143. (Currently Amended) A method for determining the initial amounts of individual species of a target gene, comprising:

- (1) amplifying a target gene and monitoring the amplification by a quantitative real-time PCR making use of a fluorescence-quenching probe labeled with a fluorescent dye at the 5'-end thereof as a primer(s);
- (2) digesting the amplified target gene using an endonuclease;
- (3) thermally modifying the obtained gene fragments into single-stranded forms

(4) detecting gene fragment(s) labeled with a fluorescent dye at the 5'-end thereof by a sequencer or HPLC by measuring a fluorescent emission in the fluorescent dye as a signaling marker;

(5) measuring the fluorescent intensity of fragment peaks detected by the sequencer or HPLC, which peaks are caused by the gene fragments,

and then, determining a composition ratio of individual fragments; and

(6) determining the initial amount of the target gene from the percentage of a change in the intensity of fluorescence occurring as a result of hybridization of the nucleic acid ~~probe~~ probe to and the amplified target gene; and

(7) determining the initial amounts of individual species of the target gene by multiplying the initial amount of the target gene by said composition ratio of individual fragments,

wherein said fluorescence-quenching probe is labeled at a phosphate group or a 5'-OH group of a ribose or deoxyribose which is obtained by dephosphorization of the 5'-end, and has a base sequence designed such that, when the probe hybridizes at the end portion thereof to the target nucleic acid, at least one G (guanine) base exists in a base sequence of the target gene at a portion 1 to 3 bases apart from end base of the target gene hybridized with the probe.

144. (Currently Amended) The method of Claim ~~s 110 or 111~~ 110, wherein said polymorphous analysis is T-RELP.

145. (Previously Presented) The method of Claim 144, wherein the amplified target gene is digested by making use of at least one endonuclease selected from Bso FI, Hha I, Hph I, Mn1 I, Rca I, Alu I and Msp I.

146. (Previously Presented) The method of Claim 144, wherein said restriction endonuclease is at least one endonuclease selected from Rca I, Alu I and Msp I.

147. (Previously Presented) The method of Claim 145, wherein said endonuclease is Hha I.

148. (Previously Presented) The method of Claim 143, wherein the gene fragments obtained by said digestion are analyzed and determined using a sequencer or HPLC.

149. (Currently Amended) The method of Claims ~~110 or 111~~ 110, wherein said quantitative real-time monitoring PCR method makes use of a nucleic acid probe labeled with a fluorescent dye and a quencher, wherein the fluorescent dye is one dye selected from FITC, EDANS, Texas red, 6-Joe, TMR, Alexa 488, Alexa 532, "BODIPY FL/C3", and "BODIPY FL/C6"; the quencher is one dye selected from Dabcyl, "QSY7", "QSY33", Ferrocene, methyl viologen, and N, N'-dimethyl-2,9-diazopyrenium.